

1. (Currently Amended) A substantially pure human ~~or rabbit~~ 3-phosphoinositide-dependent protein kinase that phosphorylates and activates protein kinase B $\alpha$  at a rate substantially the same as the rate wild-type PDK1 activates protein kinase B $\alpha$ , wherein the protein kinase comprises amino acids 83 to 342 and 450-550 of SEQ ID NO:1.

2. (Currently Amended) A substantially pure human ~~or rabbit~~ 3-phosphoinositide-dependent protein kinase according to claim 1, wherein the protein kinase has a molecular weight of about 67 kDa as determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis.

3. (Currently Amended) A substantially pure 3-phosphoinositide dependent protein kinase having the amino acid sequence as shown in SEQ ID. NO:1 with from 2 1 to 4 conservative substitutions therein and wherein the protein kinase activates protein kinase B $\alpha$  at a rate substantially the same as the rate wild-type PDK1 activates protein kinase B $\alpha$ .

4. Canceled

5. (Currently Amended) A substantially pure human ~~or rabbit~~ protein kinase according to Claim 1 wherein the protein kinase is a phosphatidylinositol-3,4,5-triphosphate-dependent protein kinase.

6. (Original) A protein kinase according to Claim 1 which activates protein kinase B $\alpha$  in the presence of the D-enantiomer of sn-1-stearoyl-2-arachidonyl phosphatidylinositol 3,4,5-trisphosphate but does not activate protein kinase B $\alpha$  in the presence of the L-enantiomer of the said phosphatidylinositol 3,4,5-trisphosphate.

7. (Original) A protein kinase according to Claim 1 which is activated by the D-enantiomer of *sn*-1,2-dipalmitoyl phosphatidylinositol 3,4,5-trisphosphate or *sn*-1,2-dipalmitoyl phosphatidylinositol 3,4-bisphosphate but is not activated by the L-enantiomers of the said phosphatidyl inositol phosphates.

8. (Original) A protein kinase according to Claim 1 which is not activated by phosphatidylinositol 3,5-bisphosphate or phosphatidylinositol 4,5-bisphosphate or phosphatidylinositol 4-phosphate or phosphatidylinositol 3-phosphate or inositol 1,3,4,5-tetrakisphosphate.

9. Canceled

10. (Withdrawn) A polynucleotide encoding the protein kinase of claim 1.

11. (Withdrawn) A polynucleotide according to claim 10 which contains no introns.

12. (Withdrawn) A vector comprising the polynucleotide of claim 10.

13. (Withdrawn) A host cell comprising the polynucleotide of claim 10.

14. (Withdrawn) A host cell comprising the vector of claim 12.

15. (Withdrawn) A method of making a 3-phosphoinositide-dependent protein kinase that phosphorylates and activates protein kinase B $\alpha$  or a fragment or fusion of said protein kinase, the method comprising culturing a host cell of claim

13 which expresses said 3-phosphoinositide-dependent protein kinase and isolating said 3-phosphoinositide-dependent protein kinase from said host culture.

16. (Withdrawn) A method of isolating a protein kinase according to claim 1, the method comprising the steps of (a) obtaining material that contains said protein kinase, (b) obtaining cell free extracts from said material which contain said protein kinase, c) fractionating said cell free extract and (d) selecting a fraction from step c) which is capable of phosphorylating and activating protein kinase B $\alpha$  in the presence of a 3-phosphoinositide.

17. Canceled

18. (Withdrawn) A method of identifying a compound that modulates the activity of a protein kinase according to claim 1, the method comprising:

contacting a compound with said protein kinase according to claim 1 or a fusion protein comprising said protein kinase according to claim 1 or a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity or a fusion protein comprising said fragment and

determining whether, in the presence of said compound, phosphorylation and activation of a protein kinase B or phosphorylation of a p70 S6 kinase is changed compared to the phosphorylation and activation of a protein kinase B or phosphorylation of a p70 S6 kinase in the absence of said compound.

19. (Withdrawn) A method according to Claim 18 wherein the compound decreases the activity of the 3-phosphoinositide-dependent protein kinase.

20. (Withdrawn) A method according to Claim 18 wherein the compound increases the activity of the 3-phosphoinositide-dependent protein kinase.

21. (Withdrawn) A method according to Claim 18 wherein the compound competes with a 3-phosphoinositide.

22. (Withdrawn) A method according to Claim 21 wherein the compound prevents activation of protein kinase B $\alpha$  in the presence of phosphatidylinositol-3,4,5-trisphosphate or phosphatidylinositol-3,4-bisphosphate.

23. (Withdrawn) A method according to Claim 18 wherein the compound modulates the activity of a human or rabbit 3-phosphoinositide-dependent protein kinase by binding to the protein kinase B $\alpha$  and preventing phosphorylation and activation of protein kinase B $\alpha$  by the 3-phosphoinositide-dependent protein kinase.

24. (Withdrawn) A method of identifying a compound that mimics the effect of a 3-phosphoinositide on a protein kinase according to claim 1, the method comprising determining whether said compound activates the protein kinase according to claim 1 or a fusion protein comprising said protein kinase according to claim 1 or a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity or a fusion protein comprising said fragment so that it can phosphorylate and activate a protein kinase B or phosphorylate a p70 S6 kinase, the activation by said compound being in the absence of a phosphatidylinositol-3,4,5-trisphosphate or a phosphatidyl-inositol-3,4-bisphosphate or another 3-phosphoinositide.

25. (Withdrawn) A method according to Claim 24 wherein

the 3-phosphoinositide is phosphatidylinositol-3,4,5-trisphosphate or phosphatidyl-inositol-3,4-bisphosphate.

26. (Withdrawn) A method of screening for compounds which modulate the activity of a protein kinase according to claim 1, or a fusion protein comprising said protein kinase according to claim 1 or a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity or a fusion protein comprising said fragment, or compounds which modulate their interactions with a 3-phosphoinositide or with protein kinase B, wherein said screening comprises:

contacting a compound with the protein kinase according to claim 1 or a fusion protein comprising said protein kinase according to claim 1 or a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity or a fusion protein comprising said fragment and

selecting compounds which modulate the activity of said protein kinase according to claim 1 or a fusion protein comprising said protein kinase according to claim 1 or a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity or a fusion protein comprising said fragment.

27. (Withdrawn) A method of activating protein kinase B the method comprising contacting said protein kinase B with a 3-phosphoinositide-dependent protein kinase according to Claim 1.

28. (Currently Amended) A kit comprising substantially pure human ~~or rabbit~~ 3-phosphoinositide-dependent protein kinase according to claim 1, a fusion protein comprising said protein kinase, a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity, or a fusion protein comprising said fragment.

29. (Withdrawn) A method of activating protein kinase B comprising contacting the protein kinase B with a 3-phosphoinositide-dependent protein kinase as defined in claim 1.

30. (Previously Presented) A fusion protein comprising the protein kinase according to claim 1, a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity, or a fusion protein comprising said fragment.

31. (Previously Presented) A substantially pure 3-phosphoinositide-dependent protein kinase according to claim 1 wherein the protein kinase comprises amino acids 52-556 of SEQ ID NO:1 or amino acids 52-556 of SEQ ID NO:1 with from 1 to 4 conservative substitutions therein.

32. (Previously Presented) A fusion protein comprising the protein kinase according to claim 31, a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity, or a fusion protein comprising said fragment.

33. (Withdrawn) A polynucleotide encoding the protein kinase of claim 30.

34. (Withdrawn) A polynucleotide encoding the protein kinase of claim 31.

35. (Withdrawn) A polynucleotide encoding the protein kinase of claim 32.